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October 28, 2003

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APPLICATION NUMBER: *60/409,874*
FILING DATE: *September 11, 2002*
RELATED PCT APPLICATION NUMBER: *PCT/US03/28525*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

W. Montgomery

W. MONTGOMERY
Certifying Officer

9-12-02

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PTO/SB/16 (8-95)
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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR § 1.53 (b)(2).

Docket Number	02082	Type a plus sign (+) inside this box->	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Le	Ngoc-Anh		1779 Lavista Oak Drive, Decatur, GA 30033
Brown	Virgil	W	3208 Habersham Road, Atlanta, GA 30305

TITLE OF THE INVENTION (280 characters max)

FAT-INDUCED ACUTE RESPONSE (FIAR) A BIOCHEMICAL ASSESSMENT OF ENDOTHELIAL DYSFUNCTION

CORRESPONDENCE ADDRESS

Office of Technology Transfer, EMORY UNIVERSITY, 2009 Ridgewood Drive, Atlanta

STATE	GA	ZIP CODE	30322	COUNTRY	USA

ENCLOSED APPLICATION PARTS (*check all that apply*)

X	Specification	*Number of Pages*	14	X	Small Entity Statement
	Drawing(s)	*Number of Sheets*	0		Other (specify)

METHOD OF PAYMENT (*check one*)

	A check or money order is enclosed to cover the Provisional filing fees	PROVISIONAL FILING FEE AMOUNT($)	$80.00
X	The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number: 50-13-11		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No

☐ Yes, the name of the U.S. Government agency and the Government contract number is:

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express No. ET937041590US in an envelope addressed to:

Commissioner of Patents and Trademarks
Box Provisional Patent Application
Washington, D C 20231

on this 11th day of September 2002.

Helen G. Carter — Helen G. Carter

Date

Respectfully submitted,

SIGNATURE Mary L. Severson

TYPED or PRINTED NAME Mary L. Severson, Ph.D., Esq.

Date Sept 11, 2002

REGISTRATION NO. 34, 927

☐ Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FILING ONLY

Express Mail No: ET937041590US

EMORY UNIVERSITY

Office of Technology Transfer

Commissioner of Patents
and Trademarks
BOX PROVISIONAL PATENT APPLICATION
Washington, D.C. 20231

Re: **U.S. Provisional Patent Application**
Filed: September 11, 2002
For: "FAT-INDUCED ACUTE RESPONSE (FIAR) A BIOCHEMICAL ASSESSMENT OF ENDOTHELIA DYSFUNCTION"
Emory File No.: 02082

Dear Sir:

Enclosed for filing regarding the above-referenced patent application are:

(1) PROVISIONAL APPLICATION COVER SHEET;
(2) SPECIFICATION ;
(3) SMALL ENTITY STATEMENT
(4) AUTHORIZATION TO PAY FROM DEPOSIT ACCOUNT IN THE AMOUNT OF $80.00; AND
(5) RETURN POSTAL CARD.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-13-11.

Sincerely,

Mary L. Severson. Ph.D., Esq.
Reg. No. 34,927

Enclosures

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on this 11th day of September 2002

September 11, 2002
Date

Helen G. Carter

Emory University
2009 Ridgewood Drive
Atlanta, Georgia 30322
An equal opportunity, affirmative action university

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EXPRESS MAIL NO.ET937041590US

EMORY FILE NO: 0282

CHECKLIST FOR: File Provisional Application Under § 2.53(b)(2)

DATE PROVISIONAL APPLICATION FILED: _____

DATE PROVISIONAL APPLICATION BECOMES AUTOMATICALLY ABANDONED: _____
(12 mo after filing date)

DATE NONPROVISIONAL APPLICATION CLAIMING PRIORITY MUST BE FILED: _____
(If last day of pendencey for provisional application accurs on a Sat., Sun., or Federal holiday within the Dist. of Columbia, the nonprovisional (regular) application must be filed **prior to** the Sat., Sun. or Federal holiday)

☒ HC Prepare *Provisional Application Cover Sheet* [H:\pforms\provisio app\prov-cov.doc]

☐ If Drawing(s) are included in the application, label Drawings [H:\pforms\provisio app\draw-lab.doc] None DRAWing

☒ HC Prepare Small Entity form [H:\pforms\provisio app\se.doc]

☐ Determine if an assignment is to be filed at this time. If so, prepare both the *Assignment* and the *Assignment Recordation Form* [H:\pforms\proviso app\assign.doc and H:\pforms\proviso app\ass-rec.doc]

☒ HC Prepare Cover letter [H:\pforms\provisio app\cov-let.doc]. Also prepare separate Cover letter for *Assignment* if it is being filed [H:\pforms\provisio app\assign cov-let.doc].

☐ Determine if paying by deposit account or check; if check, prepare Provisional Application fee check: (for $80.00 small entity)

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☐ Prepare return postal card(s) [separate card needed if assignment is being filed] [if electronic, H:\pforms\provisio app\fillin.doc]

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Washington, D.C. 20231

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- ☐ Executed COM on Cover letter(s), Provisional Application Cover Sheet
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- ☐ Prepare "Post Card Monitoring Sheet" for patent file
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1. Date App Filed _____
2. 1 year date from filing to file regular US and Worldwide _____
3. 6 months from filing date to review file and send letter to inventor(s) in preparation for potential Worldwide filing _____
4. 9 months from filing date for review file and send letter to inventor(s) in preparation of Worldwide filing _____
5. Assignments filed _____

- ☐ File completed checklist in Patent file; File Patent file

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60409874.091102

Express Mail No.: No.ET937041590US

Confidential Information

Emory University
Invention Disclosure Statement

Description

1. Please list below all persons who are believed to have made significant contributions to the invention. (Note: the determination of inventorship is a legal matter and will be determined by legal counsel)

Major Contributor 1	
Name	Le, Ngoc-Anh
Social Security No.	557-88-0407
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Major Contributor 2	
Name	Brown, W. Virgil
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Campus Phone	404-235-3001
Campus Fax	404-235-3005
Pager Number	
Email Address	W.Virgil.Brown@med.va.gov
Citizenship	USA

Note: If there are additional investigators, please list on a separate sheet.

For OTT Use Only		
Publication	Yes	No
Government	Yes	No
Tech. Type		
File No.		
OTT Associate		

2. Title of Invention

60409874 . 091102

Express Mail No.: NO.ET937041590US

Fat-Induced Acute Response (FIAR) A Biochemical Assessment of Endothelial Dysfunction

3. Give a brief description of your invention. (Use additional pages and attachments if necessary).

We have observed an acute and transient reduction in the levels of circulating plasma levels of autoantibodies against an oxidized form of LDL in patients with documented CAD. This response could not be demonstrated in young, healthy individuals with no known heart disease. We also demonstrated that this response can be elicited only with certain oil formulations that are enriched in polyunsaturated fatty acids. We have now recently demonstrated that, in patients with documented CAD, therapy with Zocor can modulate this acute response. We believe that this test can be used to identify individuals with diseased endothelium as an early sign of atherosclerosis and can be used to monitor the efficacy of therapy.

4a. Key words:

Cardiovascular Disease | Endothelial Dysfunction | Oxidized LDL

5. From the description, expand on novel and unusual features. How does the invention differ from present technology? What problems does it solve or what advantages does it possess? (Use additional pages if necessary.)

1. This is the only biochemical test for the detection of atherosclerotic endothelium.
2. Therapeutic intervention known to improve the endothelium can be assessed by the test.
3. The acute fat test is based on a very physiological challenge (20 grams of fat) and should have clinical and pathological importance.
4. In theory, this test should be able to detect presence of inflammation in the endothelium, a feature characteristic of early atherosclerotic lesions before any occlusion and permanent damage could have been caused.

6. If not indicated previously, what are possible uses for the invention? In addition to immediate applications, are there any indirectly related applications? (Use additional pages if necessary.)

Same as in Item 5.

7b. Does the invention have disadvantages? Can they be overcome? How? (Use additional pages if necessary.)

We are currently using a commonly available oil formulation for the test, it is possible that we can identify a number of specific fatty acids that are responsible for the response and can prepare capsules containing pure mixtures of these fatty acids with a longer shelf-life. The shelf-life of the current test formulation is 1 week in the refrigerator or 3 months in the freezer.

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8. Attach sketches, drawings, photographs, or other material that help illustrate the description. Rough artwork, pencilled grants, etc. are acceptable as long as they tell a clear and understandable story.

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Please list any of your publications (including abstracts, posters, news releases, etc.) in print or anticipated.

Which of the following apply to this invention?

	Yes	Date	No
Publication			
Oral Presentation			
Poster Session			
Disclose to Company Representative			
Other Public Dissemination			

Give title of your publication or presentation, the publisher, and the publisher's phone number if known. Attach copies o draft manuscripts, pre-prints, reprints, etc. Please furnish any additional manuscripts/abstracts prior to submission for publication.

Evidence for the in vivo generation of oxidatively modified epitopes in Patients with Atherosclerotic Endothelium Metabolism 49(10): 1271-1277; 2000.

Please identify any companies you believe may be interested in evaluating your disclosure for commercial development.

Execution Of Assignment By Inventor

A. Execution by Inventor(s)

___ I hereby solemnly swear and affirm under oath that I am the only inventor of this invention and that I have not knowingly omitted the inclusion of any other inventor(s) besides me.

✓ We hereby solemnly swear and affirm under oath that we are the only inventors of this invention and that we have not knowingly omitted the inclusion of any other inventor(s) besides us.

Signature of Major Contributor(s) — Date 8/16/2002

Confidential Information

Execution by Witnesses:

The invention was disclosed and explained to me by the major contributor(s) whose signature(s) appear(s) above

Signature of Witness(s) Date

B. Assignment

Agreement to Assign the Invention to Emory University

I/we, THE UNDERSIGNED investigator or joint investigators of the invention disclosed in this invention report and have read and understand this Invention Disclosure Statement. I/we request Emory University (the "University") to consider this disclosure under the Emory University Patent Policy (the "Patent Policy"). I/we agree to comply with all terms of the Patent Policy and that my/our sole compensation for such compliance, assignment of the Invention to the University and all other agreements made herein shall be that compensation provided for in the Patent Policy. I/we authorize the University to review this disclosure, to independently assess actual inventorship, to evaluate its commercial potential, to submit non-confidential disclosure agreements with third parties, to enter into confidential disclosure agreements with third parties providing for disclosure of the Invention, to file patent applications disclosing and claiming the Invention and to license and otherwise commercialize the Invention.

I/we agree to cooperate with the University and sign all patent applications, assignments and any other papers deemed necessary by the University to enable it to apply for, obtain, maintain, protect, license and assign patents covering the Invention and to confirm the University's ownership of all rights in the Invention.

Major Contributor 1:
Signature [signature] Date 8/16/2002
Typed Name LE, Ngoc Anh

Major Contributor 2:
Signature _____ Date _____
Typed Name _____

Major Contributor 3:
Signature _____ Date _____
Typed Name _____

Major Contributor 4:
Signature _____ Date _____
Typed Name _____

Concepts for Patent Filings related to PUFA Oxidation by Arterial Wall

From: W. Virgil Brown, MD
Professor of Medicine

Nhoc Anh Le, PhD
Associate Professor of Medicine

Warren Davis, MD
Assistant Professor of Medicine

1. Polyunsaturated fatty acids given orally can be used to diagnose active arteriosclerosis.
2. Polyunsaturated fatty acids given intravenously as a triglyceride or phospholipid emulsion can be used to diagnose active arteriosclerosis.
3. .....can be used to monitor vascular disease as an indicator of the success of any treatment including the reduction of blood cholesterol, blood pressure or cigarette smoking.
4. The fall in endogenous antibodies against various oxidatively damaged proteins or lipids following the administration of PUFA can be used as a diagnostic test for active arteriosclerosis.
5. The generation of various products of oxidation of lipids in the blood of persons following the intravenous or oral administration of PUFA can be used to diagnose arteriosclerotic vascular disease.
6. The generation of various products of oxidation of lipids in the blood of persons following the intravenous or oral administration of PUFA can be used to determine the success of treatments for arteriosclerotic vascular disease.

Fat Induced Antibody Reduction (FIAR) as a Test For Active Vascular Disease: A Dose-Response Study.

Principal Investigator:
W. Virgil Brown, MD
Professor of Medicine
Emory University School of Medicine
Chief of Medicine and Primary Care
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Mail Code 111
1670 Clairmont Road
Decatur, Georgia 30033

Co-Investigators:
Warren Davis, MD
Assistant Professor of Medicine
Emory University School of Medicine

Nhoc Anh Le, PhD
Associate Professor of Medicine
Director of Emory Lipid Laboratory

Matthey Harris, MD
Postdoctoral Fellow
Department of Medicine
Emory University

Amount Requested: $100,000

Summary of Protocol:
Vascular dysfunction accompanies the active disease process of atherosclerosis and is determined in large part by endothelial cell dysfunction. This dysfunction may result from the induction of a highly oxidative state within the endothelial cells overlying active atherosclerotic lesions. The generation of reactive oxygen species (ROS) such as superoxide anion (0_2^-) and hydrogen peroxide (H_20_2) change intracellular metabolism and generate a reactive oxidative state on the cell surface. Polyunsaturated fatty acids that exist in cell membranes and in circulating lipoproteins are particularly vulnerable to oxidation. Previous studies have found antibodies to oxidized lipoproteins, specifically malondialdehyde (MDA) modified low-density lipoproteins that seem to be ubiquitous among humans. In patients with proven atherosclerosis, these antibodies fall significantly after an easily oxidized substrate is provided in the form of a polyunsaturated fat meal, while the antibody levels remain normal in healthy controls. We are proposing a study to better define this phenomenon so that it may be used as a test in the future to not only identify patients with endothelial dysfunction but also monitor their response to appropriate treatment. We are also planning on measuring the actual products of oxidation, such as lipoperoxides and MDA, generated by the polyunsaturated fat meal using two different methods.

Specific Aims: **1)** To determine the dose and type of fat to optimize the fat Induced Antibody Reduction (FIAR) test by comparing, over the full dose range, the relative fall in plasma antibodies to malondialdehyde (MDA) modified LDL following the feeding of Omega 6 (linoleic acid) containing fat and that containing predominantly Omega 3 (eicosapentaenoic acid) fat in patients with active atherosclerotic vascular disease. **2)** To correlate the fall in anti MDA-LDL IgG after oral polyunsaturated fatty acid (PUFA) administration directly with the generation of malondialdehyde modified proteins by measuring these proteins in the plasma. **3)** To measure other oxidation products such as lipoperoxides and correlate these levels temporally with the fall in antibodies to MDA-LDL. **4)** To validate the FIAR test as a future clinical tool to target patients with endothelial dysfunction and to judge successful treatment of active arteriosclerosis.

Background: Vascular dysfunction accompanies the active disease process of atherosclerosis and is determined in large part by endothelial cell dysfunction (1-4). This impairment is due, in part, to the generation of ROS, such as superoxide anion (0_2^-), peroxynitrite or hydrogen peroxide (H_2O_2), which create a highly oxidative environment. ROS are produced by the endothelium and other vascular cells under the influence of cytokines as a major causative component of the atherosclerotic process. Proteins and lipids in this environment are altered in a variety of ways. One class of biological compounds that are most vulnerable to oxidation is the group of polyunsaturated fatty acids that exist in cell membranes and in circulating lipoproteins. This reaction generates a series of reactive aldehydes that form covalent bonds with other molecules in the immediate environment. One of the most common reactions appears to be that with the epsilon amino group of the lysine side chains, which exist as components of nearby proteins. This generates a new surface epitope on the protein, which is immunogenic. The occurrence of circulating antibodies to MDA-lysine side groups on proteins seems to be ubiquitous among humans and is commonly found in animals as well (5-8).

Previous studies have shown that antibody levels to malondialdehyde (MDA) modified LDL drop after a fatty meal in subjects with coronary artery disease while they remain stable in normal subjects (10). The decrease in antibody levels is maximum at approximately 2 hours after the meal and returns to baseline by 4-6 hours (See Attachment 1). Further investigations have shown that only fats that are rich in polyunsaturated fatty acids (PUFA) will cause the drop in antibody levels while meals composed of saturated fats or monounsaturated fats will not cause this effect (11). The source of polyunsaturated fats in studies in this laboratory has been exclusively from safflower oil. This oil is rich in triglycerides containing linoleic acid (2 double bonds beginning with the omega 6 carbon atom.)

Although the suppression of anti-MDA-LDL is almost certainly due to the development of newly modified proteins by a sudden massive generation of malondialdehyde from the transport of polyunsaturated fatty acids (PUFA), it remains important to document this more fully. Therefore in this series of experiments, we propose to measure the MDA proteins directly by a commercial ELIZA. This has been a difficult and variable assay, possibly due to the rapid clearance of these modified proteins as immune complexes. In 20 to 24 patients, we expect the average values to confirm an increase of their MDA proteins in the plasma during the first 2 hours after the oral PUFA administration.

There are also methods to quickly measure other oxidation products of the PUFA such as lipoperoxides. This methodology requires immediate bedside assay and is a

research procedure with little clinical potential. However, we believe correlating this test will also help confirm the theory of the mechanism underlying the FIAR test.

It should be noted that one reason the FIAR test has great potential as a clinical test is that the IgG concentration is very stable and the concentration can be assessed days or weeks later if the plasma is protected from simple evaporation. The test can be done on frozen plasma after years of storage as documented in this laboratory.

Drugs that reduce blood cholesterol (Statins) and antihypertensive agents (ACE Inhibitors) are known to reduce the oxidative potential of the endothelium. Preliminary evidence from this laboratory also indicates that the fall in antibodies to MDA-LDL after a PUFA meal can be markedly reduced by statin therapy with values approaching those seen in normal individuals (See Attachment 2).

This strongly suggests that this observation can be developed into a test for detecting patients with endothelial dysfunction and hence active atherosclerotic disease. The immediate corollary is that this test can then be used for evaluating successful treatment of active atherosclerosis. The most appropriate fat and the optimum dose of this fat should be defined if this type of assessment is to be used effectively. In clinical trials of efficacious treatments of the causative factors in arteriosclerosis, we may be able to determine early in the course of treatment which patients have altered the pathophysiology of arteriosclerosis and will ultimately have fewer clinical events. The test might then be used for adjusting treatments to achieve a "normal result" that predicts the expected improvement in long-term outcome. To date, no clinically applicable test is available for this purpose.

Research Design:

Study Population:

A total of 24 subjects with active vascular disease will be recruited. They will be screened by a history and physical exam, measurements of lipid profile and routine blood chemistries to include CBC, TSH, free T4, ALT, AST, CPK, CRP, BUN, creatinine, glucose, hemoglobin A1c (if diabetic), urinalysis for protein and glucose. First preference will be given to subjects with coronary artery disease. Exclusions will include the use of lipid lowering or high dosè angiotensin II- altering (or blocking) drugs within 3 months of the study. Because of the limitations in finding subjects with coronary artery disease that are not on lipid lowering drugs or other vascular active drugs, we will also accept subjects with high coronary disease risk ratings as determined by Framingham Risk Assessment including both hard and soft risk predictors (12). We would expect these individuals to have diseased endothelium in spite of the absence of previous history of heart disease. We will also screen people using measures of arterial compliance (VASOGRAM) and endothelial reactivity (ENDOGRAM). *Detailed information available upon request.*

Inclusion Criteria:

1. Ambulatory Men of age >40 years old of all ethnicities.
2. Ambulatory Women of age > 50 years old of all ethnicities.
3. LDL-C > 130 mg/dl but <250 mg/dl.
4. Triglycerides <300 mg/dl.
5. One or more of the following criteria:
    a. Framingham Risk Assessment (Hard Risk) predictive of ≥ 20%
    b. Framingham Risk Assessment (Soft Risk) of ≥ 20% in males and ≥15% in females.
    c. Type II Diabetes mellitus using ADA criteria

d. Convincing evidence of atherosclerotic vascular disease within any vascular field.
e. Abnormal VASOGRAM and/or abnormal ENDOGRAM

Exclusion Criteria:

- Any acute event caused active arterial disease within the prior three weeks.
- Uncontrolled hypertension (Systolic BP > 150 or diastolic BP>95).
- Abnormal thyroid function (TSH or free T4 outside the normal range for lab.).
- Uncontrolled Type II diabetes (HgbA1C>9%) or Type I Diabetes.
- Active inflammatory disease including liver, renal, or autoimmune disorders.
- Use of any active lipid lowering medication within three months.
- Use of higher doses of any angiotensin II altering or blocking medication within the past 3 months.
- Institution of medications during the previous two months for treatment of diabetes, thyroid disease, high blood pressure, acute infectious process, ovarian failure, or other disorders as judged by the investigators as possibly altering the results of the experiment.
- Consumption of fish oil supplements.
- Consumption of large doses of substances of significant "antioxidant potential" within two months (Vitamins C, E, beta-carotene, etc.).

Recruitment of volunteers:

Two groups of up to twelve study subjects will be recruited primarily from the Atlanta VAMC and the Emory Clinic patient population but will not be limited to these populations.

- Linoleic acid dose response:
  Group 1 will receive three meals containing Safflower oil at increasing doses and one meal containing only the eicosapentaenoic acid (Ethyl-EPA).
- Eicosapentaenoic dose response:
  Group 2, will consume three meals containing Ethyl-EPA at increasing doses and a single meal containing safflower oil.

These four fat containing meals will be administered to each volunteer in Groups 1 and 2 at intervals of approximately one week. The fat content will be increased with each fat feeding to provide 8, 16, or 32 grams of PUFA either from safflower or from Ethyl EPA. One of the studies will contain 16 grams of the alternative oil (safflower or Ethyl-EPA). **Safflower oil** is a mixture of triglycerides containing 74.6% linoleic, 14.2% oleic, 4.3% palmitic, 2% stearic acids and small amounts of other fats. Thus the 8 grams of linoleate containing meal will require 10.7 grams of safflower oil, etc. **Ethyl-EPA** represents the ethyl ester of eicosapentaenoic acid purified from fish muscle oil. The Ethyl-EPA capsules are a product of Laxdale Limited in Scotland. It is stated by the manufacturer that the capsules are 99% the ethyl ester of eicosapentaenoic acid.

Non-invasive tests of arterial compliance (VASOGRAM) and endothelial function will be performed before and two and one half (2 ½) hours after the fatty meal. Endothelial function (ENDOGRAM) is assessed by measuring pulsatile flow in the arm before and after a 5-minute period of ischemia.

Procedures by visit:

- Screening Visit: Eligibility determined. Consent form signed if eligible, fasting blood sample drawn, medical history reviewed. Physical exam performed. A VASOGRAM

and ENDOGRAM procedure will be done if volunteer is qualified for the study. The total blood withdrawn will be approximately 20 ml.

- Study Visit 1: Blood will be drawn (20 ml) before the fat is consumed. Blood will also be drawn at 1, 2, 3 and 4 hours after eating the fat. This visit should last about 5 hours. A VASOGRAM and ENDOGRAM procedure will be done before the fat is consumed and at two and one half (2 ½) hours after the fat is consumed. The first meal will contain approximately 10.7 grams of safflower oil (Group 1) or 8 (1 gram) Ethyl-EPA capsules.
- Study visits 2 and 3 will be scheduled approximately one week apart. The procedure will be the same as in visit 1 except that 22 grams of safflower will be consumed at visit 2 and 40 grams at visit 3 for those in Group 1. For Group 2, Ethyl-EPA capsules will be consumed, 16 capsules at visit 2 and 32 at visit 3.
- Study visit 4 will involve the consumption of Ethyl-EPA capsules, 16 grams of fat, if volunteer is in Group 1. If a participant in Group 2, the meal at visit 4 will be the "shake" containing 22 grams of safflower oil to provide 16 grams of linoleic acid.
- The visits will be in the Lipid Research Center of the Atlanta VA Medical Center or the General Clinical Research Center at Emory University Hospital.
- The total blood withdrawn for the entire sequence of tests over the five-week period will be less than 500 ml.

Methods:

1. All lipid, lipoprotein, and apolipoprotein measurements will be performed in the Emory Lipid Research Laboratory. This laboratory is a participant in the NHLBI/CDC Lipid Standardization Program.
2. MDA proteins will be done in the Emory Lipid Research Laboratory using a commercially available kit.
3. The FIAR (Fat Induced Antibody Response) test requires the measurement of the human plasma IgG antibodies to MDA-LDL using a "sandwich ELISA" assay consisting of MDA-LDL attached to plastic plates, onto which the plasma is added and incubated allowing the specific antibodies to bind. These are then quantitated by a rabbit anti human IgG linked to alkaline phosphatase as the signaling molecule. This assay will be performed in the Emory Lipid Research Laboratory (10).
4. IgG-LDL immune complexes will be measured in the Emory Lipid Research Laboratory using an ELISA consisting of specific antibody to human LDL attached to plastic plates. After incubating human plasma on such plates, the quantity of attached human IgG is measured by the addition of rabbit-alkaline phosphatase complex (10).
5. Lipoperoxides will be measured using the Free Oxygen Radical Monitor made by INCOMAT MED, GmbH, Germany. (13)
6. **The VASOGRAM,** recorded by an FDA approved device supplied by the Vasocor Corporation of Charleston, SC, will be used to measure compliance (VASOGRAM) in the arteries of the thigh and leg and endothelial function in the arm (ENDOGRAM). This evaluation consists of non-invasive measurements that use blood pressure cuffs that are controlled by a computer to make the measurements.
7. The Standardized test meal consists of a fruit shake prepared with frozen orange juice, nonfat yogurt, sugar and the appropriate amount of oil.

Data Analysis and Sample Size Estimate:
The Fat Induced Antibody Response will be estimated by the area under the curve method with each test meal. Each participant in this study will serve as his and her own control for the 4 postprandial studies using different type and amount of fat. This is expected to minimize variability in response due to biological factors. Available data would indicate that the gender and ethnic background of the participant have no effect on the fat-induced autoantibody response. Assuming a 10% measurement error for the determination of the levels of antibodies in the plasma, we expect to detect a 11.14% difference in AUC between any two test meals by paired 2-tailed t-test with a 90% power from a study population of 12 participants.

References:

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2. Nabel EG, Selwyn AP, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. J AM Call Cardiol. 1994; 16: 349-356
3. Treasure CB. Klein JL. Weintraub WS. Talley JD. Stillabower ME. Kosinski AS. Zhang J. Boccuzzi SJ. Cedarholm JC. Alexander RW. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. NEJM 1995;332(8):481-7.
4. Wilkinson I, Qasem A, McEniery C, Webb D, Avolio A, Cockcroft J. Nitric Oxide Regulates Local Arterial Distensibility in Vivo. Circulation 2002; 105:213-217.
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Fat-Induced Acute Response as an assessment of endothelial dysfunction: Effect of statin therapy.

We have previously reported that patients with atherosclerotic endothelium exhibit an acute and transient reduction in the levels of autoantibodies against malondialdehyde (MDA)-modified LDL following the consumption of a standardized liquid formula containing polyunsaturated fatty acids (Metabolism 49: 1271-1277; 2000). This acute reduction was not observed in young healthy controls with normal endothelium. Our hypothesis is that the interactions of intestinally derived chylomicrons with the diseased endothelium promote the oxidative modification of dietary polyunsaturated fatty acids resulting in the formation of MDA epitopes in the vascular space. Statins as an efficient class of agent for the reduction of LDL have also been suggested to reduce oxidation stress on the arterial tree. The present study is designed to address whether therapy with simvastatin (40mg/day) can affect the fat-induced acute response (FIAR) in patients with documented CAD. Thirteen individuals with documented CAD have been recruited and preliminary data on the first 6 patients are available at this time. Plasma lipids were determined by enzymatic methods. The levels of autoantibodies against MDA-LDL were determined by ELISA as previously described using plasma samples collected at baseline, 1, 2, 3, and 4 hours after the consumption of the fat-containing drink. Consistent with earlier studies, FIAR was demonstrated in all subjects with documented CAD with the maximum reduction of 15% in AAb levels occurring at 2-hr after the test meal. After 4 months on therapy, LDL levels were reduced by an average of 32.1%. FIAR was significantly reduced after simvastatin therapy for all time points in all studies completed to-date. The mean normalized AAb levels were 0.88 vs. 0.98 at 1 hr, 0.86 vs. 0.93 at 2 hr, 0.98 vs. 0.98 at 3 hr and 0.95 vs. 0.96 at 4 hr. The area under the 4-hr displacement curve was statistically significant ($p < 0.002$) using two-tailed paired t-test. The present data would suggest that statin therapy may have a direct effect on endothelial dysfunction by reducing the propensity of the arterial wall to generate oxidatively modified epitopes and that FIAR may provide a useful biochemical assessment of endothelial dysfunction.

We claim:

1. A method to diagnose arteriosclerotic vascular diseases, said method comprising the steps of:

   (a) giving polyunsaturated fatty acids orally or
   (b) giving polyunsaturated fatty acids intravenously as a triglyceride or phospholipid emulsion;
   (c) detect the fall in endogenous antibodies against various oxidatively damaged proteins or lipids following the administration polyunsaturated fatty acids.

2. A method to monitor and manage vascular disease treatment and oxidative stress, said method comprising of:

   (a) giving polyunsaturated fatty acids orally or
   (b) giving polyunsaturated fatty acids intravenously as a triglyceride or phospholipid emulsion;
   (c) detect the presence of various products of oxidation of lipids in the blood of persons following the administration polyunsaturated to determine the success of treatment for arteriosclerotic vascular diseases or status of oxidative stress.

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Filed or Issued:
For:

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. 1.9(f) and 1.27(d)) – NON PROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: EMORY UNIVERSITY
ADDRESS OF ORGANIZATION: 2009 Ridgewood Drive
Atlanta, GA 30322

TYPE OF ORGANIZATION:

☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION LOCATED IN ANY COUNTRY
☐ AN ORGANIZATION OF THE TYPE DESCRIBED IN SECTION 501(c)(3) OF THE INTERNAL REVENUE CODE OF 1954 (26 U.S.C. 501(c)(3)) AND EXEMPT FROM TAXATION UNDER SECTION 501(a) OF THE INTERNAL REVENUE CODE (26 501(a))
☐ ANY NONPROFIT SCIENTIFIC OR EDUCATIONAL ORGANIZATION QUALIFIED UNDER A NONPROFIT ORGANIZATION STATUTE OF A STATE OF THIS COUNTRY (35 U.S.C. 201(i))
☐ ANY NONPROFIT ORGANIZATION LOCATED IN A FOREIGN COUNTRY WHICH WOULD QUALIFY AS A NONPROFIT ORGANIZATION UNDER THE ABOVE PARAGRAPHS (2) or (3) IF IT WERE LOCATED IN THIS COUNTRY

I HEREBY DECLARE THAT THE NONPROFIT ORGANIZATION IDENTIFIED ABOVE QUALIFIES AS A NONPROFIT ORGANIZATION AS DEFINED IN 37 C.F.R. 1.9(E) FOR PURPOSES OF PAYING REDUCED FEES UNDER §41(A) AND (B) OF TITLE 35, UNITED STATES CODE, WITH REGARD TO THE INVENTION ENTITLED
"Fat-Induced Acute Response (FIAR) A Biochemical Assessment of Endothelia Dysfunction"
by inventor(s)
described in

☒ the specification filed herewith
☐ application serial no. filed
☐ patent no. issued

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or a nonprofit organization under 37 C.F.R. 1.9(3). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. 1.27)

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 C.F.R. 1.28(b)).

I hereby declare that all statements made herein or my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Mary L. Severson, Ph.D., J.D.
TITLE IN ORGANIZATION: Assistant Vice President and Director
Office of Technology Transfer
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SIGNATURE _____ DATE Sept. 11, 2002

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